AN EXAMINATION OF INTRASEASONAL VARIATION IN THE INCIDENCE OF MELANISM IN PEPPERED MOTHS, *BISTON BETULARIA* (GEOMETRIDAE)

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ABSTRACT. We examined daily catch records of peppered moths (*Biston betularia*) taken over a forty-year period to determine whether melanic and pale forms of this species fly at the same or different times within seasons. We also compared the emergence rates of the two forms from reared broods to determine whether differences in developmental rates might contribute to intraseasonal flight patterns. Although melanic phenotypes develop slightly faster than their typical siblings in some laboratory broods, the field records show no consistent pattern for one phenotype being more common than the other early or late in the same summer. The work is discussed in the context of industrial melanism for which this species is the classic example.

Additional key words: *cognataria*, f. *carbonaria*, industrial melanism, f. *swettaria*.

Industrial melanism is a familiar textbook example of observable evolution brought about by natural selection. The term pertains to increases in the frequencies of genetically determined melanic versus pale phenotypes in populations living in habitats modified by regional industrial development and urbanization. The phenomenon has been well documented in many species of Lepidoptera; however, attention has focused primarily on the peppered moth, *Biston betularia* (L) (Geometridae). The broad aspects of the subject have been reviewed recently by Majerus (1998), Sargent et al. (1998), and Grant (1999).

The corpus of experimental work to date is consistent with the interpretation that selective predation on the moths by birds is the primary, though not exclusive, force driving the changes in the frequencies of peppered moth phenotypes (Majerus 1998). Kettlewell (1955, 1956) provided the first quantitative evidence that birds eat the different color phases of peppered moths according to their conspicuousness on different backgrounds. His mark-release-recapture experiments also demonstrated that the melanic phenotypes fared better than the pale forms in soot-blackened woodlands; whereas, the pale forms fared better than the melanics in unpolluted woodlands.

Kettlewell (1973) entertained other possibilities beside selective predation that might contribute, at least in part, to the high incidence of melanism in moth populations living in the vicinities of British industrial centers. He speculated that larvae developing in the early part of the summer feed on leaves that are less contaminated by industrial pollutants than are the older leaves that larvae feed on later in the summer.

From his observations of larval developmental rates, he proposed that pale peppered moths, as fast developers, avoided pollution, and/or that the melanics, as slow developers, "may be capable of getting rid of toxic substances." He cited no reference to support his statement that "slow feeding and a capacity for excreting noxious materials has been demonstrated . . . outside the Lepidoptera" (Kettlewell 1973:85).

Unfortunately, Kettlewell's developmental analysis of peppered moths was limited to one brood that was partially consumed by mice, and a second brood that provided "no corroboration of the earlier results." He also acknowledged that he could provide no evidence from samples of wild populations that industrial melanic forms change in frequency during the flight period within single seasons. However, he did discuss intraseasonal changes in phenotype frequencies for several other moth species which show what he called "ancient" (=stable polymorphism) melanism. For example, pale *Amathes glareosa* increase late in the season (the melanics appear early on); whereas, *Cleora repandata* melanics increase, relative to pale forms, as the season progresses.

In analyses of seasonal catch records of other moth species polymorphic for melanic forms, Bishop et al. (1978) concluded that melanic *Gonodontis bidentata* emerged later than pale forms, and Sargent (1983) reported slight increases in melanism during the second half of seasons in *Phigalia titea*. As only field data were available from these studies, clear distinctions between selection on the adults and developmental differences in emergence schedules between the phenotypes were not possible. Equally problematical, S. Poitout (cited

TABLE 1.   June (=A) and July (=B) catch records of *B. betularia* phenotypes (Mel. = melanics, T+I = typicals and f. *insularia*) at Caldy Common between 1959 through 1998. The phenotypic distributions during June and July of each year are compared by *G*-tests of independence.

Year	Mel	T+I	G	Year	Mel	T+I	G	Year	Mel	T+I	G	Year	Mel	T+I	G
59A	174	15		69A	349	25		79A	387	111		89A	52	114	
59B	90	4	1.46	69B	216	20	0.67	79B	111	23	1.72	89B	13	40	0.91
60A	178	11		70A	658	68		80A	379	115		90A	36	68	
60B	34	2	—	70B	112	10	0.18	80B	103	40	1.30	90B	15	35	0.33
61A	161	18		71A	109	8		81A	118	46		91A	102	337	
61B	240	12	4.45*	71B	79	11	1.75	81B	178	65	0.08	91B	139	355	2.93
62A	159	17		72A	45	3		82A	43	15		92A	227	750	
62B	588	38	2.56	72B	202	26	1.26	82B	45	19	0.22	92B	32	111	0.05
63A	725	67		73A	102	14		83A	219	111		93A	41	146	
63B	185	18	0.03	73B	181	21	0.21	83B	226	133	0.88	93B	22	65	0.37
64A	238	25		74A	99	14		84A	172	105		94A	39	196	
64B	335	37	0.03	74B	178	22	0.14	84B	43	32	0.56	94B	26	87	2.01
65A	361	40		75A	78	16		85A	189	144		95A	33	149	
65B	70	7	0.06	75B	155	20	1.60	85B	271	256	2.34	95B	13	66	0.11
66A	163	18		76A	129	29		86A	213	198		96A	5	78	
66B	110	8	0.93	76B	242	46	0.05	86B	169	225	6.78**	96B	12	106	1.12
67A	123	10		77A	68	12		87A	141	201		97A	12	165	
67B	243	21	0.02	77B	360	50	0.46	87B	41	61	0.03	97B	5	56	—
68A	209	26		78A	91	28		88A	44	66		98A	14	113	
68B	216	26	0.01	78B	205	31	5.92*	88B	29	40	0.07	98B	12	87	0.07

*p < 0.05
**p < 0.01

by Bishop et al. 1978) showed in laboratory strains of *Spodoptera exigua* that melanic genotypes had a longer total development than non-melanic genotypes; thus melanic individuals tended to emerge from pupae later than the non-melanics, but no field data were reported.

The above studies serve to illustrate that generalities are not obvious regarding developmental differences and/or in intraseasonal variations in flight patterns between melanic and pale forms of polymorphic moth species. Therefore, we have analyzed the flight patterns in *B. betularia* directly, and compared the emergence rates of melanic and pale phenotypes. To determine if melanic and pale phenotypes fly at different times during the summer, we examined the daily catch records of peppered moths taken at a single location over a forty-year period. To assess potential differences in emergence schedules, we recorded the eclosion sequences of melanic and pale phenotypes from three large broods produced from controlled crosses.

MATERIALS AND METHODS

Field studies of intraseasonal flight patterns.
Biston betularia were collected near Caldy Common,

West Kirby, England over a period of 40 years, beginning in 1959. The original purpose of the study was to assess frequency changes in the melanic, pale, and intermediate phenotypes over the course of years; these annual changes have been reported elsewhere (see Clarke et al. 1985, 1994, Grant et al. 1996, 1998). To determine if the different phenotypes fly at different times during the same season, we reexamined the daily catch records for each of the 40 years moths were trapped at this location.

Each season a mercury vapor (MV) light trap was operated nightly from 1 June through 31 July. When virgin females were available, an assembling (pheromone) trap was also used. No difference in the proportions of the phenotypes caught by one trapping method or the other has ever been observed (Clarke et al. 1994), but the incidental use of the assembling trap does increase the total catch size. For our analysis, therefore, we subdivided each season into early and late halves based on calendar date (June versus July) rather than by the mid-point (median) of the total numbers of the moths caught within seasons. We then tabulated the numbers of each phenotype caught during the first and second halves of each season. The null
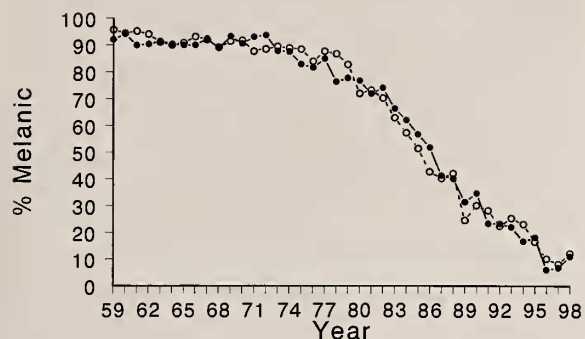
Fig. 1. The decline in the frequency of melanic *B. betularia* between 1959 through 1998 at Caldy Common, West Kirby, England, plotted from June samples (solid symbols) and from July samples (open symbols).

hypothesis is that there should be no difference in the percentage of melanics caught in the early and in the late samples taken during the same summer.

Laboratory experiments on differential emergence. To determine if differential emergence (time to eclosion) of melanic versus pale phenotypes exists, we crossed known heterozygous melanic *B. betularia cognataria* to their pale siblings. (The expected ratio of pales to melanics among the progeny from such matings is 1:1.) The stock material was produced by a melanic female crossed to a pale male, both of which were caught at the same location in Pennsylvania in 1996, and the crosses used in our experiments were made using their progeny which emerged in the spring of 1997.

In all, three sets of siblings were crossed, and their broods were subdivided to avoid overcrowding. The caterpillars were housed inside plastic "garbage cans" and were provided a continuous supply of fresh leaves from a single, large Chiswell crab apple tree (*Malus* spp.). The caterpillars pupated "at will" in moistened beddings in the bottoms of their containers. The pupae were then stored in containers lined with moistened paper towels. All storage containers were checked daily to remove newly emerged adults until the experiment was terminated several weeks after emergences ceased entirely and no living pupae remained. For each brood, each moth to emerge was identified by phenotype and sex and the date of its emergence.

Results

Field studies. From 1959 through 1998, 18,255 *Biston betularia* were collected near Caldy Common.

The year-to-year sample sizes varied widely, ranging between 122 to 1120, with an average annual catch of 456 ± 257. The early and late halves (June vs. July) of seasons also varied widely with respect to sample sizes, with mean catches at 259 ± 206 and 197 ± 143, respectively. The differences between the early and late sample sizes, though large, are not statistically significant by paired samples t-tests (t = 1.622, df = 39, p = 0.113), nor by the non-parametric Wilcoxon's signed-ranks test (p = 0.226).

The complete set of catch records, subdivided by early (A = June) and late (B = July) catches, are summarized by phenotype in Table 1. In the table, the melanics (f. *carbonaria*) are separated from the other phenotypes (pale = f. *typica* or "typicals" and intermediates = f. *insularia*). The intermediates have remained rare at Caldy Common (for complete data through 1993 see Clarke et al. 1994), therefore the combined category (T+I) is essentially "typical" (pale), or non-melanic.

Sample sizes permitting, the numbers of melanics and non-melanics collected during the first half and second half of each season were compared using 2 × 2 contingency G-tests of independence. The G statistics are listed in Table 1. Of the 38 comparisons made, only three showed significant differences in the phenotypic proportions between the early and late halves of the same season; in two instances the melanics increased significantly (p < 0.05) in the second half of the season, and in one instance the melanics declined very significantly (p < 0.01) during the second half of the season. In 35 of the 38 comparisons, no significant differences in the proportions of melanics between the early and late subsamples within seasons were observed.

To test for the possibility that small but consistent differences might exist within seasons (differences too slight to be detected by G-tests), we analyzed the entire 40-year record by Wilcoxon's signed-ranks test. The null hypothesis is that the differences in the percentages of melanics collected during first and second halves of seasons are random. The data show that the percentage of melanics increased during the second half of summers 23 times, and decreased 17 times (Fig. 1), but the differences are not significant by Wilcoxons signed-ranks test (p = 0.55).

Between 1977 and 1997 the annual incidence of melanism declined rapidly at Caldy Common (Fig. 1), and for several years during this same period, there appeared to be a short run of seasons in which the percentage of melanics decreased in the second half of summers compared to the first half. However, the pattern is not consistent throughout the period of rapid annual decline in melanism, and the differences in

Table 2. Early and late emergences from reared broods of *B. betularia cognataria* from crosses expected to produce 1:1 ratios of melanic and pale (typical) phenotypes. The phenotypic distributions between the early and late groups within each brood are compared by *G*-tests of independence.

Brood			Melanics	Typicals	G
A	Early		53	46	
	Late		49	36	0.31
B	Early		30	23	
	Late		16	29	4.37*
C	Early		27	27	
	Late		23	30	0.47

**p < 0.05

melanic frequencies between first and second halves of seasons are not significant by Wilcoxons signed-ranks test (p = 0.82).

Differential emergence. We recorded the emergence date, sex and phenotypes of all adults produced by the three crosses of heterozygous melanics mated to their pale, homozygous siblings. Eclosion patterns within broods generally begin with one or a few adults emerging from puparia on the initial day, then the number of daily emergences increases quickly and peaks a few days later, after which the daily emergences tail off until finally many days may separate the stragglers from the main group and from each other. The null hypothesis of our experiment is that the difference in emergence sequence within broods is not related to the color phenotypes; therefore, the proportions of melanics and typicals should be the same in the first half of a brood to emerge as in the second half.

We divided each brood into early and late groups using the median. All of the moths emerging up to and including the day the first half of the total emergences for the brood was reached were assigned to the early group, and all of the moths emerging after that day were assigned to the late group. Table 2 lists the early and late emergences by phenotype (melanic and typical) for each of the three broods. The numbers of melanics and typicals emerging during the early and late periods are compared within broods by 2 × 2 contingency *G*-tests of independence. The *G* statistics are also listed in the table. In brood B, melanics are significantly more common in the early than in the late group (p < 0.05), but no significant emergence differences are apparent in the other two broods.

Discussion

The data from Caldy Common provide little support for the idea that the different phenotypes of *Biston betularia* fly at different times of the season. That three of the 38 contingency tests indicated significant intraseasonal differences between the phenotypic proportions is consistent with type-I error rates expected when large numbers of statistical comparisons are made (Sokal & Rohlf 1981). Furthermore, only two of the three observed significant deviations from random expectations were skewed in the same direction which further supports that these exceptional years can be attributed to chance. Of these three exceptional years (1961, 1978, 1986), only 1986 shows a very pronounced deviation in phenotypic proportions between the early and late halves of a season, and in this instance the significant decline in the proportion of melanics occurred during the brief run of intraseasonal declines in melanism that occurred during those years of the steepest declines in the annual incidence of melanism. Here, at least, it seems reasonable to suggest that selection at the adult stage rather than developmental differences in emergence schedules might account for the drop in melanism within that season.

Differences in emergence schedules between melanic and pale peppered moths are slight, at best. Of the three broods we examined, only one showed significantly accelerated emergence of the melanics versus their typical siblings. Different broods, no doubt, emerge throughout the normal season of several months, and if developmental differences, however slight, between melanics and pale forms are consistent, then, in the absence of offsetting selection at the adult stage, we should expect to observe a consistent bias favoring melanics earlier in the summer. Clearly this did not happen over the 40 years of observations at Caldy Common.

Our developmental studies in the laboratory employed the North American subspecies, *B. betularia cognataria*, and our field studies centered on the British subspecies of peppered moths, *B. betularia betularia*. Direct comparisons between the two must be qualified. American "pale" or typical forms are generally much darker than British typicals; however, the melanics (called f. *carbonaria* in Britain and f. *swettaria* in America) are phenotypically indistinguishable, and are caused by alleles at the same locus (Grant & Clarke in prep.). The melanics, both in America and Britain, have also shown parallel increases (Owen 1962) and decreases (Grant et al. 1995, 1996) in frequencies associated with environmental modifications related to industrial development and urbanization. The reductions in melanism on both continents are now widespread (Grant et al. 1998).

The phylogenetic relationship (Rindge 1975, Clarke et al. 1993), and the genetics of melanism in these subspecies (West 1977, Grant & Clarke in prep.), and the

ecological events coincidental to the parallel evolutionary changes in phenotype frequencies (Grant et al. 1996, 1998) combine to suggest that we are observing the same basic phenomenon in peppered moth populations on both sides of the Atlantic. While we must remain cautious in extrapolating what we learn from the study of one subspecies to draw conclusions about the other, we also recognize that what we learn from one subspecies might be instructive in our study of the other. In this instance, there again appears to be agreement: No consistent intraseasonal flight pattern differences between pale and melanic British peppered moths are apparent, nor are the differences in emergence schedules of pale and melanic American peppered moths consistent among broods.

Our study does not address one of Kettlewell's (1973) suggestions that differences in developmental rates might exist among the larvae of different genotypes that produce pale and melanic adult phenotypes. No clear relationship between emergence rates between melanic and pale peppered moths and the time the forms spend as larvae has ever been established; therefore, our study on emergence sequences does not bear directly on the question of the seasonal conditions early and late larvae might experience. However, there is evidence that *B. betularia cognataria* is bivoltine (Owen 1962, Manley 1981); therefore, larvae of those genotypes producing melanic and pale adults in the first versus the second generations of summers must endure late and early summer conditions, respectively, as they feed and develop. Yet, there is no evidence that the proportions of melanic and pale adults change consistently between the first and second broods of summers (Owen 1962, Manley 1981). These observations do not support Kettlewell's speculations that selection at the larval stage influences melanism at the adult stage.

We do not consider this a "negative results" paper. There has been much speculation about the relevant ecological factors and the putative selective agents responsible for industrial melanism, and now its decline (recently critiqued by Majerus 1998, Sargent et al. 1998, and Grant 1999). The occasional but recurring query that intraseasonal variation in the frequencies of the forms may offer some insights can, in our view, be laid to rest. Part of the process in identifying what is important from what is not involves the process of elimination. We hope we are making progress.

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